

JUN 10 2004
OFFICIAL**PATENT****IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

APPLICANT : HIGGINBOTHAM et al.
 SERIAL NO : 09/871,183
 FILED : May 31, 2001
 TITLE : METHODS AND COMPOSITIONS FOR EFFICIENT GENE
 TRANSFER USING TRANSCOMPLEMENTARY VECTORS

Grp./A.U. : 1636
 Examiner : Guzo, D.
 Conf. No. : 9947
 Docket No. : P04580US01

PETITION TO ACCEPT PHOTOGRAPHS AS DRAWINGS
(37 C.F.R. 1.84(b)(2))

Commissioner of Patents and Trademarks
 Washington, D.C. 20231

Dear Sir:

1. Petition is hereby made to accept photographs in this case.
2. Three (3) sets of photographs were previously submitted June 23, 2003.
3. The reason why photographs are necessary in this case is as follows:

Figure 5 depicts the human adrenocortical xenograft tumors in nude mice. (A) Female nude mice were injected subcutaneously into the right flank with 10×10^6 SW-13 cells. Prominent tumor nodules formed intra- and/or subcutaneously and after 6-7 weeks

CERTIFICATE OF MAILING/TRANSMISSION (37 CFR 1.8(a))

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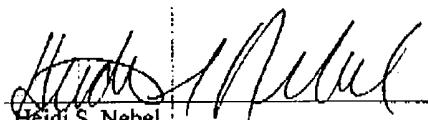
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 Heidi S. Nebel

metastases occurred. (B) Whole-body volume reprojection image of an adrenal tumor-bearing mouse after injection of 18F-FDG. Primary tumor (T) and metastasis (M) are visible. Two data collections were required to visualize the whole animal (H=heart, B=brain, HA=Harderian glands) (C and D) Haematoxylin/Eosin staining of primary tumor (PRIM) and metastasis (META) from Figure 5B. Histological studies revealed multilobulated primary tumors with an adrenal like texture. Primary tumors had a pseudo-capsule (PC) and were highly vascularized (V, vessel) and abundantly exhibited mitotic nuclei (arrowheads). Tumor cells within penetrating vessels (arrows) underline the metastatic capacity of the tumor. The histological characteristics of the tumor were identical in the metastases (40 x).

Figure 7(B) transmission electron micrographs demonstrating nuclear viral particles *in situ*. Transcomplementing transduced tumors were examined for newly assembled viral particles 96 h after transduction. *De novo* assembling of viral particles inside the nucleus is a characteristic feature of virus production and indicative for replication. The figure depicts characteristic adenoviral particles with circular electron dense structure and halo as nuclear inclusion in a SW-13 tumor cell.

Figure 9 depicts analysis of cell death in transcomplementing transduced tumors. Figure 9A depicts free 3'-ends of DNA were labeled with digoxigenin-marked deoxyUTP, subsequently immunodetected with anti-DIG-peroxidase and visualized with AEC. Red stained single nuclei and cell clusters throughout the specimen suggestive of apoptosis. Stained foci were frequently found adjacent to sites of injection and to areas demonstrating viral transduction. Figure 9B shows the same section demonstrating areas viral transduction by green fluorescence of the transgene product (20 x magnification).

Figure 10 depicts ultrastructural analysis of cell death in tumors Xenografts transduced with transcomplementing adenoviral vectors AVC2.TK plus H5.d1014. Tumors exhibited mitochondria with tubulo-cristular structure typical of adrenocortical cells. (A) Cells showed apoptotic signs such as vacuolization of the nucleus (N) and cytoplasm, but only a slight condensation of chromatin and fragmentation of nuclei. The mitochondria (M) exhibited in this figure remained morphologically unaffected, indicating a grossly intact

metabolism in these cells (bar=0.1 μ m). (B) Higher magnification of the inset from figure 10A demonstrating intranuclear viral particles (arrows) indicative of viral replication.

Figure 11 depicts DU 145 spheroids were transduced with Ad GFP + Ad Null or Ad GFP + Ad *dl*1011 and subjected to confocal microscopy.

Figure 12 depicts DU 145 Cells were transduced with various ratios of Ad GFP + Ad Null or Ad GFP + Ad *dl*1011 and photographed at 20 hrs.

Figures 13 (a-c) depict HCT 115 tumors were injected 1×10^8 PFU with Ad GFP + Ad Null or Ad GFP + Ad *dl*1011 or Ad GFP + Ad *dl*1010 or Ad GFP + Ad *dl*1014 or Ad GFP + Ad *dl*1020 and tumors photographed at the indicated times.

Figures 14 (a-c) depict HTC 116 tumors were injected 1×10^8 PFU with ADGFP + Ad Null or AdGFP + Ad *dl*1011 or AdGFP + Ad *dl*1010 or AdGFP + Ad *dl*1014 or Ad GFP + Ad *dl*1020 and tumors photographed at the indicated times.

4. A check in the sum of \$130.00 accompanied the previously filed Petition to Accept Photographs as drawings filed June 23, 2003. It is not believed any additional fees are due, however, please charge Account 26-0084 for any fee deficiency, or credit said account for any overpayment.

Respectfully submitted,



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